

Serial No.: 09/981,289

Filed: October 15, 2001

the paper copy thereof serve to place this application in a condition of adherence to the rules 37 C.F.R.

§ 1.821-1.825.

Please direct any calls in connection with this application to the undersigned at (415) 781-1989.

Respectfully submitted,

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Dated: \_\_\_\_\_

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Paragraph beginning at page 3, line 18, has been amended as follows:

- Figure 6A (SEQ ID NO:1) depicts the nucleotide sequence of the histidine tagged wild-type TNF- $\alpha$  molecule used as a template molecule ~~form~~ from which the mutants were generated. The additional 6 histidines, located between the start codon and the first amino acid are underlined.–

Paragraph beginning at page 3, line 21, has been amended as follows:

- Figure 6B (SEQ ID NO:2) depicts the amino acid sequence of wild-type TNF- $\alpha$  with an additional 6 histidines (underlined) between the start codon and the first amino acid. Amino acids changed in the TNF- $\alpha$  mutants are shown in bold. –

Paragraph beginning at page 4, line 13, has been amended as follows:

- Figure 12 (SEQ ID NOS:3-8) depicts trimerization domains from TRAF proteins. –

Paragraph beginning at page 21, line 26, has been amended as follows:

- The TNF- $\alpha$  proteins may be from any number of organisms, with TNF- $\alpha$  proteins from mammals being particularly preferred. Suitable mammals include, but are not limited to, rodents (rats, mice, hamsters, guinea pigs, etc.), primates, farm animals (including sheep, goats, pigs, cows, horses, etc); and in the most preferred embodiment, from humans (the sequence of which is depicted in Figure 6; SEQ ID NO:2). As will be appreciated by those in the art, TNF- $\alpha$  proteins based on TNF- $\alpha$  proteins from mammals other than humans may find use in animal models of human disease. –

Paragraph beginning at page 25, line 16, has been amended as follows:

- The variant TNF- $\alpha$  proteins and nucleic acids of the invention are distinguishable from naturally occurring wild-type TNF- $\alpha$ . By “naturally occurring” or “wild type” or grammatical equivalents, herein is meant an amino acid sequence or a nucleotide sequence that is found in nature and includes allelic variations; that is, an amino acid sequence or a nucleotide sequence that usually has not been

intentionally modified. Accordingly, by "non-naturally occurring" or "synthetic" or "recombinant" or grammatical equivalents thereof, herein is meant an amino acid sequence or a nucleotide sequence that is not found in nature; that is, an amino acid sequence or a nucleotide sequence that usually has been intentionally modified. It is understood that once a recombinant nucleic acid is made and reintroduced into a host cell or organism, it will replicate non-recombinantly, i.e., using the in vivo cellular machinery of the host cell rather than in vitro manipulations, however, such nucleic acids, once produced recombinantly, although subsequently replicated non-recombinantly, are still considered recombinant for the purpose of the invention. Representative amino acid and nucleotide sequences of a naturally occurring human TNF- $\alpha$  are shown in Figure 6 (SEQ ID NOS:1-2). It should be noted that unless otherwise stated, all positional numbering of variant TNF- $\alpha$  proteins and variant TNF- $\alpha$  nucleic acids is based on these sequences. That is, as will be appreciated by those in the art, an alignment of TNF- $\alpha$  proteins and variant TNF- $\alpha$  proteins can be done using standard programs, as is outlined below, with the identification of "equivalent" positions between the two proteins. Thus, the variant TNF- $\alpha$  proteins and nucleic acids of the invention are non-naturally occurring; that is, they do not exist in nature.—

Paragraph beginning at page 28, line 4, has been amended as follows:

— Thus, the variant TNF- $\alpha$  proteins of the present invention may be shorter or longer than the amino acid sequence shown in Figure 6B (SEQ ID NO:2). Thus, in a preferred embodiment, included within the definition of variant TNF proteins are portions or fragments of the sequences depicted herein. Fragments of variant TNF- $\alpha$  proteins are considered variant TNF- $\alpha$  proteins if a) they share at least one antigenic epitope; b) have at least the indicated homology; c) and preferably have variant TNF- $\alpha$  biological activity as defined herein.—

Paragraph beginning at page 29, line 26, has been amended as follows:

— In one embodiment, the nucleic acid homology is determined through hybridization studies. Thus, for example, nucleic acids which hybridize under high stringency to the nucleic acid sequence shown in Figure 6A (SEQ ID NO:1) or its complement and encode a variant TNF- $\alpha$  protein is considered a variant TNF- $\alpha$  gene.—

On page 61, immediately preceding the claims, the enclosed text entitled "SEQUENCE LISTING" was inserted into the specification.

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